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The First International Congress on Forensic Pathology

Brussels, 20-21 July 1957

The final arrangements for this Congress (initiated by the South African Medico-Legal Society) have now been completed. It will be held at the Palais de Justice in Brussels on Saturday 20 July and the scientific sessions will be continued in Ghent on Sunday 21 July in the Department of Forensic Medicine of the University of Ghent.

The following organizations are the sponsors of the meeting to date:

The College of American Pathologists.
The American Society of Chemical Pathologists.
The American Academy of Forensic Sciences.
The Association of Chemical Pathologists.
The British Association in Forensic Medicine.
Societe de Medecine Legale de Belge.
The South African Medico-Legal Society.

Included in the subjects for discussion are strangulation, firearm wounds, the role of the chemist in forensic pathology, and a model medico-legal investigation of homicide.

Selected short papers of special interest will also be read. Papers should be forwarded in good time so as to reach the Honorary Secretaries by 1 June 1957.

The following arrangements have so far been approved:

On Friday, 19 July, at 9 p.m. there will be a reception by the President at the Hotel Astoria, to enable those attending the meeting and their friends to meet.

On Saturday, 20 July, in addition to the scientific sessions there will be an informal dinner (lounge suits) which will include guests.

On Sunday, 21 July, the meeting will continue at Ghent and transport by road will be arranged from Brussels. At the conclusion of the scientific sessions the meeting will terminate at Bruges with dinner where associate members and guests will rejoin the party (an inclusive charge for transport—Brussels—Ghent—Bruges—Brussels—Lunch (Ghent) and Dinner (Bruges) has been arranged). Associate members and guests may also visit Bruges, have lunch in Ostend and rejoin the party at Bruges

for dinner (an inclusive charge for transport—Brussels—Ghent—Ostend (lunch)—Bruges (dinner)—Brussels has been arranged.

Those wishing to take part in the meeting should apply (forwarding the appropriate money) to Dr. Francis E. Camps.

Cheques should be made payable to *The International Meeting of Forensic Pathology.*

Accommodation. Application for accommodation may be made to the Havas Agency, Brussels.

The cost of the Meeting will be as follows:

A. Registration fee for members, including the Programme with abstracts of papers ... ... £2 (280 Frs.)

B. Registration fee for associate members ... ... 10s. (70 Frs.)

C. Visit of members to Ghent and Bruges with lunch at Ghent and dinner at Bruges (without wines and gratuities) ... ... £2 6s. (324 Frs.)

D. Visit of associate members to Ghent-Ostend-Bruges including lunch at Ostend and dinner at Bruges (without wines or gratuities) £2 10s. (352 Frs.)

E. Official dinner, including wines ... ... £2 2s. 6d. (300 Frs.)

The Secretary Conveners are:

Prof. Dr. F. Thomas,
Department of Forensic Medicine,
23, Kluyskensstraat,
Ghent, Belgium.

Dr. Francis Camps,
Department of Forensic Medicine,
London Hospital Medical College,
London, E.1, England.

Estimation of the Time of Death by Medical Criteria

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One of the most important opinions required at the conclusion of a forensic autopsy is the answer to the question, 'When did death occur?' It must be admitted that this estimation cannot be made with mathematical accuracy; that it has necessarily to be fixed within the limits of probability; that these limits are wider the longer the interval between death and the examination of the body; and, further, that more reliable information may be available from events occurring at known times in and around the locus as, e.g. the time of delivery of a letter, than may be expected from purely medical observations of certain post-mortem changes.

In a provocative article in regard to the estimation of the time of death by the cooling rate and rigor mortis in particular, Shapiro[1] pleads for a re-examination of the 'many tenets held dear by the uninquiring mind for generations' and decries the 'peremptory adhesion unto authority' re-iterated in edition after edition and textbook after textbook.

The question does not end with the fixing of the limits of probability. It often happens that an opinion may be required whether these limits could *possibly* be extended one way or the other, in order to cover thereby some important item of fact. In a tropical climate such as ours, where post-mortem changes in general and putrefaction in particular are rapid, the reliable assessment of these limits is commensurately more difficult.

We have undertaken the investigation of those phenomena by which we are usually guided in establishing the time of death. This study was begun over 2 years ago by the examination of a series of bodies under conditions which were standardized as far as possible.

The degree of standardization of the conditions of experiment is indicated by the following facts:

1. The bodies were those of 41 condemned prisoners who were judicially executed in Ceylon.

2. The experiments were carried out at the ordinary room temperature prevailing over a period of 2 years.

3. They were restricted to those of men in normal health (confirmed by subsequent post-mortem examination) who had lived under identical conditions (type of food, physical exercise, time of meals, etc.) for a considerable period of time preceding execution.

4. Execution was by hanging at the same time of the day (8 a.m.) in all cases.

5. The bodies were laid on a table only after the pulse and heartbeat had ceased (by palpation and auscultation).

6. The examination of the upper cervical cord through an incision and the removal of the corneae for corneal grafting were carried out in the execution chamber immediately after the body was brought down.

7. Observations were carried out under the same conditions in the University laboratory in every case (except in the case of 5 bodies where of necessity the investigation was

conducted in the prison mortuary).

We embody in this report the facts observed at experiment and our conclusions from an analysis of those facts. Our conclusions have been correlated, wherever possible, with our field work. Where, however, experimental observation has not been possible, in the instances referred to in our report, our facts have been restricted to our field work.

We discuss in this report:

1. The cooling rate.
2. Rigor mortis.
3. Eye changes.
4. Gastro-intestinal contents.
5. Putrefaction.
6. Maggots.

The Cooling Rate

A full and detailed report of our work on this subject has already been published.[2] We have, therefore, restricted ourselves here to a brief statement of observations and inferences with regard to the post-mortem rectal temperature readings of experimental bodies. Since the publication of this work our conclusions have been confirmed by the further examination of 25 similar bodies. In addition, reference is made to recordings of temperature in the liver observed at the same time as the rectal temperatures in all the bodies we have investigated.

Methods of Observation

A chemical thermometer was inserted immediately after death into the rectum to a depth of 3—4 inches. The initial temperature was read after 5 minutes. Subsequent recordings were taken at half-hourly intervals—the thermometer being left *in situ* throughout the period of observation.

Results

1. *The Moment of Death.* The pulse and heart beat ceased within 15 minutes of execution in all cases.

2. *The Initial Temperature.* This ranged between 97·8° F. and 100·8° F. with a mean of 99·6° F. (Cullumbine[3] fixes the mean rectal temperature of male adults at 99·8° F.).

3. *The Lag Period.* The fall in temperature up to three-quarters of an hour after death was hardly significant (confirming the view of Schwarz and Heidenwolf[4]). This may of course be attributable to the continuance, for a short time after death, of the metabolic and oxidative processes on which heat production depends during life.[5] In addition, it is possibly due to heat loss in the dead body being primarily dependent on the loss of heat through the skin. The sequence of heat loss as observed from visceral temperature readings would envisage a preliminary fall in the skin temperature which would be followed by a fall in the visceral temperature, heat being lost

from the internal viscera by a process of conduction via the intervening tissues.

4. The cooling rate subsequent to this preliminary lag was in accordance with the general view that the temperature drop is rapid in the earlier hours and slows up later as the temperature of the body approximates that of the room temperature, which was reached in about 22 to 24 hours after death.

5. Analysis of our results indicates that:

(*a*) The body—room temperature difference has a definite bearing on the cooling rate.

(*b*) An important additional factor, besides the generally accepted processes through which heat is lost, *viz*. radiation, conduction and convection, is the influence of evaporation on the fall of temperature in a dead body. This is in agreement with the view of Strassmann.[6] Increased evaporation tends to hasten the cooling rate. That heat is lost in the dead body by evaporation is not surprising as it is well known that heat loss does occur in the living by a passive seepage of moisture through the skin (*insensible perspiration*).[7] The human body, besides, consists of about 70—80% of water.

(*c*) In view of the limited scope of our experiments, it is not possible to draw any definite conclusions about the extent of the influence of the surface area and weight of the dead body (size factor) and the humidity of the atmosphere.

(*d*) The thick cotton overalls, in which some of the bodies were clothed, did not appear to influence significantly the cooling rate as compared with others that were examined nude.

We have utilized the information obtained from the experiments for the purpose of estimating the time of death by the cooling rate. The usual formula adopted for this purpose is:

$$\frac{\text{Normal Temperature of the Body (98·4°F.)} - \text{Rectal Temperature at the Time of Death}}{\text{Rate of temperature Fall per Hour}} = \text{Number of hours since death.}$$

The temperature of the medium is taken at the same time. In contrast with the accepted rate of temperature fall of 1·5° F. per hour adopted in temperate climates for the first 12 hours, we have found that in the low country round about Colombo an average of 0·7° F.—0·8° F. per hour gives fairly reliable results when the body is examined about 5—8 hours after death. The hourly rate of fall to be adopted must, of course, depend upon the specific circumstances of each case.

Smith and Simpson[8] recommend the taking of the rectal temperature and that of the medium over a period of hours in order to obtain the *rate* of temperature fall. This information, they say, would enable one to 'appreciate the factors which are likely to influence the cooling of the body'. The taking of several readings, as suggested, would certainly enable one to

arrive at the rate of fall over the observed period, but our observations lead us to conclude that any attempt, based on such readings, to assess the fall of temperature over the period antecedent to the period of observation, can be misleading. The main cause of error in assessing the time of death by this means is the failure to appreciate fully the effect of modifying influences—influences which must be assessed both individually and collectively.

Our study (which took into consideration the factors of convection, conduction, radiation and evaporation) has resulted in a formula which in itself *embodies the influence of these factors*. Our formula seeks to determine the time at which the body was at a given temperature antecedent to the period of observation. We have taken 99·6° F. as the rectal temperature at the time of death. The formula has now been applied to a total of 66 bodies under experimental conditions and we have found it to be accurate for all practical purposes.

We were able to demonstrate that, provided the room and atmospheric temperatures did not fluctuate materially and the body remained undisturbed during the period of cooling, the rectal temperature of the body for all practical purposes was represented by the equation:

$\alpha + \beta\theta = ke^{-\beta t}$, where

α and β are constants.

θ is the difference between the rectal temperature and the atmospheric temperature.

k is a constant dependent on initial conditions.

e is the exponential constant 2·718 . . . . and

t is the time over the period of cooling considered.

From this relation we derived the formula:

$$\frac{\log(\theta_0 + p) - \log(\theta_1 + p)}{\log(\theta_1 + p) - \log(\theta_2 + p)} = \frac{t_0 - t_1}{t_1 - t_2}$$, where

θ_0 is the difference between the rectal temperature and the atmospheric temperature corresponding to the instant of time t_0;

θ_1 and t_1 and

θ_2 and t_2 are similarly related; and

$$p = \frac{\alpha}{\beta}$$

We have suggested the use of this formula for establishing the time of death of bodies cooling under the conditions indicated above, and have stipulated 8 hours after death as the period within which reasonably accurate results may be obtained.

Using the foregoing formula and allowing for an initial cooling lag of 45 minutes, we arbitrarily arrived at a satisfactory value of 2 for p as most suitable to our climatic conditions.

Since the value for p is dependent on the influence of evaporation in addition to that of other modifying factors, it may be necessary to increase

the value for p in climatic conditions where the extent of evaporation is greater than in this country.

Temperature observations in the liver

Liver temperatures were recorded (at the same time as the rectal and atmospheric temperatures described above) by a thermometer inserted, through an incision, to a depth of about 2 inches into the liver. The thermometer was kept *in situ* throughout the period of observation. We observed that in all cases the liver temperatures were initially higher than the rectal temperatures. In most cases the liver temperatures, after a lapse of about 10 to 12 hours, tended to be about the same as the corresponding rectal temperatures. The results obtained from the liver temperature readings, when used in the suggested formula, were as reliable as those obtained from the rectal temperatures.

Rigor Mortis

Rigor mortis as a criterion in the estimation of the time of death has proved of importance in many criminal trials, but it cannot be said to be a reliable guide except within wide limits.

No effort has been made as yet to correlate our observations with the factors which are generally accepted as modifying post-mortem change, as we have attempted to do in regard to the cooling rate. We restrict ourselves in this report to factual observations and such conclusions as we consider can safely be drawn.

In the study of the onset and progress of rigor mortis in our experimental bodies we have endeavoured to obviate repeated tests being applied to one particular area. We have therefore, as far as possible, used one side of the body for the earlier tests while the other side was reserved for the later tests. The degree of rigidity has been assessed by:

1. Palpation of the muscles in a particular area; and
2. Testing the degree of movement possible in the corresponding joints.

The extent of rigor present was indicated in our charts by one to four + signs, according to its degree.

Observations on the experimental bodies have been confirmed by our field work in and around Colombo. We conclude that:

1. The onset of rigor is detected in the jaw and neck muscles within the first 2—4 hours after death.

2. From thence it *appears* in general to pass upwards to the face and downwards to the chest, shoulders and abdomen and thence to the limbs.

3. It *appears* to develop centrifugally in the upper limbs as well as in the lower limbs. Usually, though not invariably, the lower limbs appear to be involved at the same time or perhaps somewhat earlier than the upper limbs.

4. The whole body, except for the fingers and toes, is generally involved in about 7—8 hours, the stage of complete rigor in the body being reached

in about 12 hours. The small muscles of the hands and feet are the last to stiffen.

5. On the average the body is in a state of complete rigor for 12—18 hours, i.e. up to about the time when putrefaction commences.

6. Our observations were made with the body lying on its back and the arms somewhat flexed at the elbow. In this position, the elbow did not attain complete rigidity. Five to 10 degrees of flexion was possible even when the body was in full rigor.

7. The disappearance of rigor follows the order of its appearance, but it is not unusual for stiffness in the jaw and lower limbs to persist after rigor has passed off from the rest of the body.

8. The rapid progress of putrefaction after its onset 24—30 hours after death, prevented us from making observations on the period required for complete disappearance of rigor in the experimental bodies. It is our view, however, that in the average case, the time required for the total disappearance of rigor would correspond with the minimum time required for the entire body to be affected by putrefaction. The tendency for rigor to persist for longer periods in the extremities—the last to be effected by putrefaction—may be explained in this way.

9. In testing for rigor in the presence of putrefaction, it is necessary to distinguish the stiffening attributable to rigor from that due to distension of the body from putrefactive changes.

Our observations have led us to doubt that the earliest manifestation of rigor is always restricted to the region of the neck and jaw, and spreads centrifugally thereafter. We have found that rigor can appear in the knee very soon after—if not at the same time—as in the jaw muscles. In such cases the hip joints and ankles were involved earlier than the shoulders and elbows. Excluding, for the purpose of discussion, the influence on rigor of those factors modifying post-mortem change in general, we would refer to those which are generally accepted as particularly influencing the phenomenon.

1. The general development and size (Shapiro[9]) of a muscle or group of muscles; and
2. The degree of activity of such muscles before death.

Rigor, then, appears first in those particular muscles in which the appropriate conditions are satisfied, irrespective of the location of the muscles and, for the same reason, progressively extends to neighbouring muscles centrifugally from them. The ante-mortem tonus of a muscle may presumably be considered as a form of ante-mortem activity and this, we suggest, explains the usually earlier manifestation of rigor in the muscles of the jaw and the neck and its subsequent progress. If these inferences are correct one would expect the very early appearance of rigor in the muscles of the heart. Strassmann (*op. cit.*, p. 135) refers to Nystem who believes that the earliest manifestation of rigor is in the heart. It would certainly be helpful to observe the time of onset of rigor in the muscles of the heart, a study

which we might undertake later.

We suggest that the assessment of the time of death from rigor mortis be made against a background of:

1. Factors influencing post-mortem change in general.
2. Size and development of muscles.
3. Ante-mortem activity of muscles.

Gastro-Intestinal Contents

We have explored the possibility that the contents of the stomach and small intestine, up to the ileo-caecal junction, may furnish some useful information in connection with the fixing of the time of death. Our findings, both in respect of our experimental and field work, have been encouraging. This has been so even when the state of putrefaction has obviated the fixing of the time of death except within comparatively wide limits.

We have not attempted to assess the degree of digestion of the ingredients of a particular meal, but have directed our observations to the rate of progress of the head of a given meal along the course of the small intestine and to a less extent to the rate of emptying of the stomach.

It is specially helpful when the ingredients of the meal are identifiable in their course through the intestine, which is fortunately often the case with the type of food usually consumed in this country, viz. rice, string-hoppers,* curry leaves and chilli or other seed.

In our experimental bodies the time and constituents of the meals taken up to 14 hours before death were available to us. The overnight meal had been taken at 6 p.m. on the previous day and the last meal at 6 a.m. on the day of (i.e. 2 hours before) execution. In the field, our observations have been based on cases in which the nature, constituents and time of the last meal were definitely known.

Methods of Investigation

The stomach is ligatured *in situ* at both ends and ligatures are applied throughout the length of the small intestine at intervals of 1—2 feet. This prevents the passage of food from one part of the intestine to another during the subsequent examination of the intestine after cutting away the mesenteric attachments. The establishment of the continuity or otherwise of a meal from the stomach onwards is of considerable importance. The stomach and gut are then incised between the ligatures and the quantity and nature of their contents noted at each successive stage. The presence or absence of disease throughout the gastro-intestinal tract and the state and nature of the contents of the ileum and the large intestine which might suggest changes in the rate of emptying, are also noted.

* A steamed preparation of rice flour, similar to riced potato, in the form of cakes.

Results and Conclusions

Our observations in general indicate that the rate of progress of a meal would depend on:

1. *The Nature of the Meal.* A liquid meal progresses faster than a semi-solid meal and a semi-solid meal faster than a solid meal. In the case of what may be termed a mixed meal, where liquids and solids have been ingested in fairly equal proportions, we have observed that the liquid precedes the solid part of the meal in the intestine by about 1—3 feet.

2. *The Stomach Contents.* We have observed that the stomach starts emptying within about 10 minutes of the ingestion of the first mouthful of a meal, and that the stomach is emptied of a small meal in a shorter time, in general, than of a large one. A full rice meal consumed by a working man in this country consisting of boiled rice, curries, etc. and amounting to about 1½ lb. *in toto*, may take as long as 7—8 hours to be completely expelled from the stomach.

3. *The Contents of the Small Intestine.* The head of a solid meal progresses along the small intestine at the rate of about 6—7 feet per hour. It reaches the ileo-caecal junction in about 3—3½ hours, where there may be a stagnation for a further hour or so.

In the application of these results to field work, the following difficulties may be met with in regard to information of:

1. *The Time of a Meal.* This may not always be quite definitely known. But even so, a fairly reliable deduction may be possible where the habits of the individual are available or may be inferred from those of the community in general.

2. *The Quantity of the Meal.* The absence of this information need cause little difficulty for it is the progress of the head of the meal in the small intestine that is of essential importance, and not the quantity of the last meal.

3. *An Interval between Two Meals.* This is usually indicated by a gap in the continuity of the intestinal contents. When, however, a separate and subsequent meal has been ingested before the stomach is completely emptied of a previous meal, there would be unbroken continuity in the intestinal contents from the stomach to the ileo-caecal junction. The time of death may, in these circumstances, especially if the 2 meals consisted of the same ingredients, be gauged from the time of ingestion of the first meal. Interpretation is more accurate as the head of the meal approximates the ileo-caecal valve, because the progress of the meal in the upper reaches of the intestine are, in general, rather faster than in the more distal portions.

Eye Changes

Our observations have necessarily been restricted to our field work, as the corneae in our experimental bodies were removed very soon after death for corneal grafting.

Pupillary Changes. The iris is composed of both constrictor and dilator fibres, of which the former are the stronger. Langworthy and Ortega[10] have shown that constriction of the pupil during life is dependent on the turgescence of the blood vessels of the iris, and dilatation of the pupil on anaemia of these vessels.

After death the tone of the musculature is lost and the pupil dilates to a position of equilibrium (Smith and Simpson, *op. cit.*, p. 173). Full dilatation may, perhaps, not be possible on account of some remaining constrictor tone and some remaining vascularity of the iris. We have observed that about 5—7 hours after death there is a partial constriction of the pupil, which we attribute to rigor mortis. At about 10—12 hours the pupil is constricted to its maximum which, however, can by no means be described as 'pin point'. In about 15—18 hours after death the pupil will once again be found in semi-dilation, which may be attributed both to the passing off of rigor and also to the anaemia of the vessels of the iris consequent on the previous constriction in rigor and the gravitation of the blood from hypostasis.

Clouding of the Cornea. In about 12 hours after death the cornea takes on a milky cloudiness which becomes more marked as time elapses. This cloudiness may be explained by the hydration of the cornea after death. During life the transparency of the cornea depends on its state of under-hydration.[11] This state is maintained by the efficient functioning of the epithelial layer on the outside, and the endothelium on the inside of the cornea. On death these 2 barriers are no longer efficient and hydration of the cornea occurs, with ensuing and gradually increasing opacity. Owing to the avascularity of the greater portion of the eye, it in general and the cornea in particular may be expected to resist putrefaction more than other tissues.

In cases where death has been protracted and especially where the deceased has been for some time unconscious or moribund, a haziness appears which Sivasubramaniam[12] attributes to an 'ante-mortem exposure keratitis' from desiccation of the superficial epithelial layers of the cornea. This, however, can be easily removed by rubbing the eyelids gently over the surface of the cornea. Another source of error is the 'cloudiness' due to the rucking up of Descemet's membrane as a result of a fall in intraocular pressure. This may occur before or after death and manifests itself as a series of more or less radially arranged parallel white striations in the cornea. Rubbing the cornea will not remove this.

Sivasubramaniam (*op. cit.*) states that humidity and atmospheric temperature influence this post-mortem cloudiness. He has been able to secure corneae for grafting before the cloudiness occurs up to a maximum of 14 hours after death, under conditions associated with a lower atmospheric temperature and a lower atmospheric humidity than those which obtain in Colombo. In Colombo these changes may therefore be expected earlier.

We have found it to occur in the average about 12 hours after death. This post-mortem change we have found to occur whether there is closure or not of the eyelids after death.

Our deductions are made as follows: The transparency or otherwise of the cornea is estimated by the degree to which it is possible to make out the outline of the pupil and the pattern of the iris. Semi-dilated pupils and clear corneae indicate a period of about 5—7 hours after death. In the presence of corneal cloudiness, a constricted pupil would indicate about 12 hours and a semi-dilated pupil about 15—18 hours after death.

Our investigations have so far been restricted to the eyes of healthy adults as seen *post mortem*. These inferences we have found to be useful in confirming the deductions made from other factors.

Putrefaction

Our experience is that in the case of normal adults in the prime of life:

1. Putrefaction first appears as a slight bluish discoloration in one or other, or both iliac fossae, in about 24 hours.
2. The whole abdomen attains a bluish discoloration in about 36 hours.
3. Swelling of the face and trunk appears in about 36—48 hours.
4. Putrefaction involves the whole body in about 60—72 hours.
5. Loosening of the epidermal casts of the hands and feet is complete in 4—7 days after death.

Once putrefaction is complete, the estimation of the time of death has necessarily to be made within comparatively wide limits.

Maggots

In the presence of marked putrefaction, in addition to the information available from the contents of the stomach and intestine, to which reference has already been made, and which has proved of use to us even as long as 4—6 weeks after burial, we have found that further information may be afforded by the presence of maggots.

Specimens of maggots should be secured for subsequent culture. Further, specimens of the largest maggots (and pupal cases) should also be collected and fixed immediately in formalin. It is essential that, in the search for these, the ground around the body be included, where pupation often occurs to a depth of 2 feet. On this subject, we commend to the reader a valuable article by Reid.[13]

Culture of the maggots removed for the purpose should be undertaken by an entomologist for:

1. Studying the habits of the fly in question; and
2. Comparing the successive stages of maggots available from culture with the maggots fixed in formalin at the autopsy.

In one of our cases[14] it was possible to assess, by culture, the probable age of the maggots removed from the body and to confirm that oviposition

would only take place during daylight, and, in the absence of external bleeding, when putrefaction was well advanced. By deduction it may be possible to determine the probable time of death from data thus collected.

Conclusion

In submitting our findings we consider that:

1. Further investigation of those criteria available to the forensic pathologist in the estimation of the time of death is very necessary under conditions obtaining in the country concerned.

2. This is especially necessary where, owing to the exigencies of a nationalized medical service, untrained or inexperienced investigators may be entrusted with the interpretation of these criteria.

3. No apology is needed for the employment of a mathematical formula for the estimation of the time of death by the cooling rate, as the formula suggested does not involve any intricate mathematical calculations.

4. An approach to the problem of the estimation of the time of death by the cooling rate, more scientific than heretofore, has been presented.

I am grateful to Dr. P. Sivasubramaniam, Visiting Surgeon, Victoria Memorial Eye Hospital, Colombo, for helpful discussion on the eye changes after death.

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Individuality of Blood

Electrochromatophoretic Patterns of Dry Blood*

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Individuality is one of the most striking features of all natural organisms, especially Man. It is, at the same time, one of the most neglected aspects from the standpoint of research, largely because the main goal of science has ordinarily been to elucidate general laws and regularities, rather than to emphasize deviations in behaviour, structure or composition. From this standpoint, individual variation has been considered a hindrance to scientific progress, and every effort has been made to eliminate the effects of individuality, as in breeding highly uniform strains of rats and mice for experimental animals, and in treating experimental data on a broad statistical basis to uncover the rule and obscure the exceptions and variations from it. In the applied sciences such as medicine, the variations of the individual cannot be safely overlooked, and much of the detailed attention given the patient by the medical practitioner is made necessary by the fact of variation.

The science of identification, which is the basis of all scientific investigation of crime, must necessarily rest directly on the deviations of the individual from the group, because it is only by these deviations that he can be distinguished from the group. This principle is universally recognized in the case of fingerprinting, which rests on the assumption that Nature never duplicates the ridge pattern of the finger tips precisely in two individuals. The fact that extremely close similarities do exist and that the points of comparison are normally limited so that the identification from finger prints is reduced to a matter of probability, does not alter the general rule of non-duplication by Nature.

Direct personal identification is still based almost exclusively on fingerprinting, and visual recognition of the physiognomy. This situation is clearly not inherently permanent, because if the rule of non-duplication is correct, the physiological fluids, hairs, or other actual body parts that may be transferred at the time of a crime should carry definite indications of

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the individual. With respect to blood, it certainly can be stated that no two bloods would be expected to be identical except as a matter of extremely minute probability, thus placing blood fundamentally in the same category as finger prints. Consideration will show that no two persons are expected to have the same sequence, chronology or severity of diseases, and that their antibody patterns could not be identical. This virtual impossibility of identity indicates the ultimate probability of making definite identifications from an individual's blood.

Another source of individual variation is certainly the metabolic pattern, about which less can be stated at present. This would affect some components of the blood in characteristic ways which might be expected to be less temporary than the obvious rapid fluctuations accompanying dietary and other environmental variations. It also might be more permanent in nature than those variations caused by disease which have so great a diagnostic significance. Whether variations that rest on differences in metabolic patterns are ever constant through life is uncertain, but they may well have long enough constancy to yield practical significance in personal identification.

The blood groups, being transmitted through the genes and therefore permanent through life, are at present the most significant factors of individuality generally recognized in the blood. Even with fresh blood in adequate amounts, it is not possible to identify an individual positively. but the eliminative value of grouping has been increased enormously by the proliferation of grouping factors that are now recognized to exist in blood. Unfortunately, blood involved in crime is normally in the form of dried spots, stains and areas on all types of supporting surfaces. It is generally recognized that only the A, B and M factors are reliably determined in dry blood by present methods. The M factor, because of its extremely wide occurrence (about 80%) is of little value as an eliminative factor, and is not regularly determined in most practising crime laboratories. Extension of available techniques to the determination of any additional hereditary grouping factors in dry blood is greatly to be desired, and may be possible in some favourable instances.

In the search for constant and unvarying criteria for personal identification throughout life, the investigator is often blinded to the obvious fact that he rarely is concerned with identification throughout life. The time span involved in the investigation of a single crime is usually short, and any factor that is constant for that length of time, with a proper allowance for safety, will be as useful as a permanent factor. In favourable instances, the time element is actually eliminated completely. When an attacker and a victim both shed blood during the commission of the crime, as very often happens, *any difference whatever* that can be established reliably, *will distinguish the two bloods.* If, in addition, criteria can be used that are reasonably permanent during at least the period of the investigation,

these will be helpful in eliminating persons other than the actual attacker, and may establish some significant probability of his identity. To be of much value, such differences should be determinable in dried blood, whether or not they are also distinguished in fresh blood.

One such difference that has been shown to exist between the dried blood of some different persons, is the character of the protein pattern produced by paper electrophoresis or electrochromatophoresis,[1] which refers to the analytical operation of continuous electrophoresis so as to develop both the electrophoretic pattern and reveal as well any adsorption paper chromatography which may occur simultaneously.

The variations in the protein pattern of different individuals in moving boundary electrophoresis has been recognized[2] and studied. Numerous investigators have noted that individual protein patterns tend to appear also with paper electrophoresis, though little has been published on this subject. Other indications of protein individuality have been observed,[3, 4] though here again, little of a systematic nature has been published except in respect of hemoglobin, several variations of which can be distinguished reliably.[5-10] It is not to be expected that the protein pattern of dried blood would be the same as that of fresh liquid blood of the same person because of the denaturation and alteration that occurs on drying. Differences in the origin of the blood may, indeed, enhance these alterations and, if this occurs, it would add to the value of the dry blood pattern.

This investigation is to be considered as a preliminary exploration of some of these problems. The type of protein pattern obtained from dry blood is studied, as well as a preliminary investigation of the effect of age, different origin of the blood and other factors. Further work on the development of more detailed patterns, and on other factors that are involved in the determination of individual differences and their interpretation will be reported in later papers.

Experimental

All dried blood patterns were developed on the micro-electrochromatophoresis apparatus,[1] using curtains cut from Whatman # 11 filter paper. Buffer used in the apparatus was made by dissolving 8 g. sodium acetate and 1 ml. of glacial acetic acid in 1 litre to give an approximately 0.1 M solution. The paper was allowed to wet and wash before placing any sample, the sample then being added in the form of dry powder, about 0.5 mg. in quantity. This was placed in a spot at the top of the grid a little left of centre by means of a creased paper so as to avoid spreading the sample over more than a minimum area.

Current was passed through the curtain immediately after adding the sample and kept constant at 300 v. which yielded a current of about 4 to 6 ma. After about 75 minutes, a pattern was obtained which occupied most of the vertical distance of the grid. The predominant branching area was on the right side, and represented at least mainly the hemoglobin. Two other smaller areas to the left were invariably visible after staining, and in no case did the dry blood give as detailed a pattern as could be obtained routinely from fresh serum.

Staining was performed by placing the curtain in a tray containing a solution of bromphenol blue (0.03%) in 95% alcohol saturated with mercuric chloride. To develop the colour fully required about 25 minutes of agitation, after which the solution was removed and the curtain washed thoroughly in running water and dried.

For experimental purposes, three individuals in the laboratory periodically deposited fresh finger blood on slides which were dated, dried and stored. Ordinarily blood was removed from all three at about the same time so that samples of the same age would be available for comparison. Other persons placed samples similarly, but at irregular intervals.

Several samples of dry blood actually involved in criminal cases were tested. These were scraped from the surface on which they had been deposited and dried. Some were taken from cloth carrying dry deposits. Several techniques were tested for this removal, when the blood was not deposited heavily enough to be scraped from the cloth directly. The one that gave the best results was to cut out the section, place it in a micro test tube and soak it in the refrigerator in a small amount of distilled water. When the solution was well coloured, it was removed to a slide and evaporated spontaneously in air in a dry, dust-free atmosphere. The dry residue could be readily removed from the slide as a fine powder. A few samples of dry serum were tested, and one series of samples was taken from a large pool of dry blood under the head of a murder victim.

Results

Blood was studied from a total of 10 persons. Not all of them could be distinguished from one another but, in several significant instances, clear differences were shown. The three individuals on whom most studies were made were all members of the O group in the ABO system of blood grouping. The three were always clearly distinguished on the basis of the two branching bands, one on the left and the other in the centre of the pattern (Fig. 1). Individual A (Fig. 1A) was strong in the left band, weak in the middle. B (Fig. 1B) was weak in both and with about the same intensity in both. The pattern of C (Fig. 1C) showed the left band stronger than the middle but consistently less so than with individual A. Most studies were made with individual A, who provided numerous samples over a period of 14 months. No significant changes in the blood pattern were found in this period. Multiple determinations at various time intervals were also made with blood of individuals B and C, but without serial samples. (The pattern of another individual D (Fig. 1D) is shown in Fig. 1 to indicate that it is distinguishable from that of the other three.)

The middle and left bands were found to be serum protein by testing dry serum. Whether any of the heavy band on the right came from serum is not definitely known, but this band was absent or extremely weak with dry serum samples and presumably is almost all or entirely hemoglobin.

The effect of time on the blood stain tested was checked by re-running the blood from the same original sample over a period of time. Four

Fig. 1. Dried blood patterns.

persons' bloods were tested in this way. Individual A gave duplicate results after 1 year's standing, but a sample run after 14 months gave a weak pattern. B showed some falling off in protein intensity of the two most significant bands at 11 months. C was weak at 1 year. One additional sample whose date of shedding was accurately known was as good a year later as when fresh, and a second one from a different person gave excellent results after 1½ years. It appears that periods approaching 1 year of standing of a dry stain have little influence on the use of the method except that unknown factors in the storage, which have not yet been

investigated, may introduce variations. All samples used in this study were stored in the laboratory out of dust, but not sealed tightly.

Single samples of three additional persons were drawn fresh and dried in the same manner as those of A, B and C. Two of these could not be distinguished definitely from blood of individual A but were different from B or C. The third was not distinguishable from individual C, but was from A and B.

Blood from a murder victim was clearly and readily distinguished from blood of the victim's spouse. Blood of another murder victim was tested comparatively against blood of his convicted murderer who had also shed blood. The differences between the two were very marked and completely prevented confusion of the two.

In connexion with the dried blood from the pool under a murder victim's head, another effect was discovered, which requires consideration and caution. Around the outside of the dried pool was an area which was thin, while toward the centre the layer was very thick and deeply coloured. This effect was caused by coagulation of the blood followed by clot retraction. The two portions of the pool gave markedly different effects in the pattern, due to the very great concentration of hemoglobin in the thick portion, which intensified the right hand band and showed the serum proteins to be essentially absent. The thin portion, consisted almost entirely of serum exudate with so little hemoglobin as to effectively eliminate the right hand band in these patterns. This effect is to be predicted because when the clot retracts without stirring, the blood cells are retained by the clot, increasing the hemoglobin concentration enormously within the retracted clot while pressing out virtually all of the serum. With ordinary blood spots this effect does not occur because drying is too rapid to allow clot retraction, and the sample ordinarily includes all of the spot that can be removed. When blood is obtained from a large pool, it is very essential that the investigator be keenly aware of this problem.

One problem not wholly dependent on aging but related to it was encountered here, as it has often been in connexion with blood grouping and precipitin testing. Some blood samples, for reasons that are not understood, become highly insoluble in a relatively short time, and all samples tend in this direction over a longer time. One sample encountered before this study, only two weeks old, virtually refused to dissolve so that even long soaking at refrigerator temperatures never gave more than a trace of colour to the solution. One sample in this series of experiments also showed a remarkable degree of insolubility. Such samples, when placed as dry powder on the apparatus, simply fail to yield a pattern or only give a very faint one, the whole of the sample remaining at the origin. While this effect is found in only a rather small percentage of blood samples, it serves to differentiate strongly between samples when it is possible to state positively that the two samples compared have been in an identical environ-

ment for the same period of time.

One other question was tested, viz. the effect of hemolysis on the pattern obtained in dry blood. Blood was drawn from the finger tip as before, and enough distilled water added to produce complete hemolysis. The blood was then dried completely and the dry material scraped off for testing. The pattern obtained was in all respects identical with that obtained with the same blood allowed to dry without hemolysis of the cells.

This study is to be considered as a preliminary one, in which some of the factors that could influence the distinction of the blood of different individuals on the basis of the electrophoretic protein pattern of the dry blood have not been fully tested. The most important ones have been tested, and it is clear that when blood has been shed at the same time, and stored under the same environmental conditions, the method will often, though not always, distinguish clearly between the sources of more than one sample. It must be emphasized that both experience and several precautions are essential to clean-cut differentiation between bloods on the basis of this technique. Experimental variations may arise from use of different apparatus, buffer or current. To compare two bloods requires that these factors all be duplicated as precisely as possible. Sample size variations are not critical as long as they are not large, and the results are properly interpreted. Reducing the size of sample will diminish the intensity of all bands, but the relative intensity of the bands in the same pattern will still be reproduced. It is in judging this effect that experience may be necessary, and it is desirable that samples be as nearly the same size as possible.

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Acute Barbiturate Intoxication

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In the United States alone, several billion doses of barbiturates are legally prescribed by the medical profession yearly. Since barbiturates are easily synthesized and may be purchased from illegal sources, additional large quantities are consumed. The purchasers of barbiturates from illegal sources are mostly barbiturate addicts. Other countries are confronted with similar consumption problems. It may be predicted that, for a few years at any rate, the new tranquilizing agents, e.g. chlorpromazine, promazine, reserpine, etc. will somewhat decrease wholesale medication or even self-medication with barbiturates. It is beyond doubt that the availability of barbiturates presents an international problem of fatal barbiturate poisoning—the drugs being taken accidentally or with suicidal intent. The medical profession, particularly the toxicologists and the forensic practitioners, must be keenly aware of the characteristic symptoms, detection and the treatment of malonyl-urea poisoning.

Fazekas and Koppanyi[1] recently submitted a questionnaire to professors of medicine, neurology, and psychiatry in American and Canadian Universities, one of the questions being whether or not barbiturates are used promiscuously by the medical profession. The answers received were: *Yes*, 75; *No*, 29; *Yes with reservation*, 8; *No opinion*, 17. These are discouraging replies in view of the potential dangers of these drugs, not only because of the possibility of severe or fatal poisoning but also of daily consumption leading to addiction.

Symptoms

Barbiturate coma is merely a form of anesthesia (light or deep) which might be produced by any aliphatic narcotic. Therefore one encounters in this condition varying degrees of areflexia, muscular relaxation and other signs and symptoms characteristic of anesthesia. Small pupils, e.g. indicate lighter and large pupils, deep surgical anesthesia. Mydriasis, therefore, is an unfavorable sign in barbiturate intoxication, and is usually accompanied by marked decrease of the respiratory minute volume, cyanosis, and very often by a fall in blood pressure and hypothermia. Hyperthermia, which

is not infrequently seen in patients who have taken toxic amounts of barbiturates, is usually due to secondary infections, particularly pneumonia. In such cases, moist rales are discovered in the lower lung fields, which progress to bronchopneumonia with consolidation.

Gross overdoses of barbiturates kill by direct depression of the respiratory center, and death may occur within an hour after oral ingestion. Such cases seldom come to the attention of the clinician for the simple reason that the patients are dead before they are discovered. The majority of the patients do not take this absolute fatal dose, but only a fraction thereof, and may survive in an anesthetized state for 24 hours or more without any treatment. Then they die either of respiratory and/or circulatory depression, pulmonary and cerebral edema or, as already indicated, they develop pneumonia. This latter group of persons, upon their discovery, requires treatment.

I submit that there is nothing definitive in the symptomatology or in the post-mortem changes of organs and tissues that could distinguish barbiturate poisoning from other forms of coma or fatal central depression. The differential diagnosis therefore must consist of chemical identification of barbiturates in the blood and the urine during the life time of the victim, or in organs, particularly the liver and the brain, following routine autopsy. The gastrointestinal contents should be carefully saved for chemical analysis because it is always possible that small amounts of unabsorbed barbiturates, particularly when taken in tablet form, are present. Fazekas, Goldbaum, Koppanyi and Shea[2] have shown that following oral administration of phenobarbital sodium in capsule form, the maximum blood levels may not be reached before 12 hours.

Chemical Identification

A. *Blood.* Ten c.c. of blood, 10 c.c. of 10% sodium tungstate solution, and from 10—20 c.c. of 2/3 normal sulphuric acid are shaken in a separatory funnel until a chocolate-brown color is obtained. The mixture is filtered with suction, and an aliquot of the clear filtrate is shaken with 10 volumes of chloroform. The chloroform fraction is filtered through a chloroform-moistened double hard filter to eliminate suspended water particles and their solutes. The filtrate may be then tested directly or it may be concentrated over a water-bath.

B. *Tissues.* The organ or tissue is weighed, placed in a Pyrex beaker, and liquid air is poured over the tissue until it is frozen. It is then pulverized and a convenient amount of this pulverized material is weighed and placed in a separatory funnel and shaken with 10 c.c. of chloroform per g. of tissue. The chloroform extract must be filtered before it is tested. If necessary, the chloroform extract may be concentrated over a water bath. This method, however, cannot be applied to the extraction of barbiturates from the brain and spinal cord because it was observed by Koppanyi, Dille and Krop[3] that lecithins and cephalins of the central nervous system are easily soluble in chloroform and interfere with the development of color in the test for barbiturates to be described. Linegar, Dille, and Koppanyi[4] showed that the interference by phospholipids can be overcome by using the following method:

Brain tissue is frozen in liquid air and pulverized. This powdered brain tissue is shaken with chloroform, the chloroform extract is filtered and evaporated to dryness on a water

bath, and the residue taken up again in a small volume of chloroform to which acetone is added drop by drop. A heavy precipitation of phospholipids occurs in the solution. The precipitate is filtered off and acetone is added again to the filtrate to ascertain whether the phospholipids have been completely removed. The solution in which no more precipitation occurs with acetone is again evaporated to dryness, and the residue taken up with chloroform and tested. In properly treated extracts, the test can be carried out without interference.

C. *Colorimetry.* Koppanyi *et al.*[5] developed several colorimetric procedures, of which we will mention the 'micro' and the 'isopropylamine' tests.

The reagents in the micro test are 0·2% of cobaltous acetate dissolved in absolute methyl alcohol, and 0·2% of lithium hydroxide in the same solvent.

Six c.c. of the chloroform extract are divided in 3 equal parts, A, B, and C.

The cobaltous acetate reagent is added to each of the 3 test tubes, 0·05 c.c. to A, 0·1 c.c. to B, and 0·15 c.c. to C.

The tubes are shaken and the lithium hydroxide reagent is added to the tubes, 0·05 c.c. to A, 0·1 c.c. to B, and 0·15 c.c. to C. The tubes are shaken and the colors noted against a plain white background. The tubes should be observed for one minute before a final reading is made.

The reactions appearing in the different tubes with different concentrations of barbiturates are shown in Table 1.

Table 1

Concentration of Barbiturates (Mg. per c.c.)	*A* 0·05 *c.c. of Each Reagent*	*B* 0·1 *c.c. of Each Reagent*	*C* 0·15 *c.c. of Each Reagent*
0·02	Positive	Negative	Negative
0·03	Positive	Positive, fading in 30 seconds	Fades immediately
0·04	Positive	Positive	Fades in 30 seconds
0·05	Positive	Positive	Fades in about 2 minutes
0·06	Positive	Positive	Permanent for more than 2 minutes

The blue colors are faint, but quite distinct. If a blue in Tube C persists for more than 2 minutes, the concentration is above the range of the test and the chloroform extract should be diluted and the test repeated. If no color is secured, a convenient amount should be evaporated to dryness, the residue dissolved with chloroform, and the test repeated with the concentrate.

The reagents in the isopropylamine test are 1·0% of cobaltous acetate dissolved in absolute methyl alcohol, and 5·0% of isopropylamine in the same solvent.

As standards, the barbiturate is dissolved in chloroform to give solutions of 0·02%, 0·04%, 0·06%, and 0·08% concentrations, kept to avoid evaporation.

One c.c. of the chloroform extract is placed in a test tube and 0·05 c.c. of the cobaltous acetate reagent and 0·3 c.c. of the isopropylamine reagent are added. If barbiturates are present in the extract, a reddish violet color develops. This is then compared in the micro-cups of a colorimeter with the color produced under the same conditions by one of the standards.

$$\frac{\text{Reading of Standard} \times \text{Concentration of Standard (mg. per c.c.)}}{\text{Reading of unknown}} = \text{Milligrams of barbiturate per c.c. of extract}$$

Green, Veitch and Koppanyi[6] have also shown that the isopropylamine test can be adapted to spectophotometric analysis. Various concentrations of barbiturates in chloroform were analyzed in the spectrophotometer at 560 m mu and the % transmittance plotted against the concentration in mg. per 100 c.c. The color developed in this test obeys Beer's law in concentrations up to 30 mg. per 100 c.c.

Another quantititative method for barbiturate determination is Goldbaum's[7] ultraviolet spectrophotometric method of differentiation of several barbiturates.

Table 2 shows the clinical and toxic doses and comatose blood levels of the more important barbiturates. The latter were arrived at either by the Koppanyi or by the Goldbaum method. The liver usually contains greater amounts of barbiturates per gram than does the blood. The concentration of barbiturates in the blood, liver and other organs is an important consideration for determining the cause of death. In accidental or unexplained death, levels considerably lower than those indicated in Table 2 may simply mean that the individuals ingested barbiturates for therapeutic purposes, and thus suicidal or homicidal intent may be ruled out.

Treatment

The treatment of barbiturate poisoning may be *symptomatic*, *analeptic* or directed toward the *removal of barbiturates* from the body. A comprehensive symptomatic therapy, recommended by Nilsson,[8, 9] includes maintenance of a patent air-way and, when necessary, oxygen administered through the air-way, administration of parenteral fluids (2 to 3 liters every 24 hours), hypertonic solutions in cases of pulmonary edema, ascorbic acid to decrease permeability of blood vessels, antibiotics to prevent secondary infections (mainly pneumonia), frequent changing of the patient's position, and periodic slapping of the thorax to loosen bronchial secretions. Mousel[10] adds the use of concentrated human albumin for the prevention or treatment of cerebral edema.

Among the many central analeptics used, picrotoxin and leptazol have been found to be most effective in the treatment of experimental and clinical acute barbiturate intoxication. Competent and carefully supervised administration avoids the occurrence of convulsions and consequently excessively increased cerebral oxygen needs, as well as depression which may follow a convulsive seizure. Recently, Koppanyi and Fazekas[11, 12] analyzed and considered the value of analeptics in the treatment of acute barbiturate intoxication. They came to the conclusion that before embarking on intensive analeptic therapy, one should give the patient an orientation dose of 5 c.c. of 10% leptazol intravenously. If the patient wakes up or his reflexes return following this dose, the patient does not require further administration of analeptics. However, if the patient does not react to this dose or reacts only by evanescent respiratory stimulation, either leptazol or picrotoxin should be given until reflex excitability and voluntary movements return. Leptazol may be administered in doses of 500 mg. every 10 or 15 minutes; picrotoxin, following an initial dose of 25 mg., in 15 mg. every 15 or 20 minutes. There is no conclusive evidence that other analeptics, amphetamine (benzedrine), coramine, disodium succinate and

caffeine exert a live-saving action in barbiturate poisoning. In fact, as Reed, Driggs, and Foote[13] have shown, caffeine and amphetamine may cause fatal arrhythmia (tachycardia) when given in doses exceeding 6 g. and 300 mg. per 24 hours, respectively.

It should be emphasized that the use of central analeptics in barbiturate intoxication is an example of pure physiological antagonism—these drugs do not increase the metabolism or excretion of barbiturates.*

Recently, several methods were proposed to increase the elimination of barbiturates. Cutting and Koppanyi[14] employed massive intravenous infusions of isotonic glucose or saline solutions in volumes from one-fourth to three-fourths the body weight in cats and dogs. This method of treatment produced (*a*) shortening of the duration of narcosis from average anesthetic doses of barbital and phenobarbital from one-third to one-half that of controls; (*b*) doubling of the early elimination of these drugs in the urine. Animals receiving 2 or 3 fatal doses of barbital recovered completely under this treatment. It was concluded:

'The mode of antidotal action of massive infusions is believed to be dependent upon the ability of the infused fluids to dissolve a part of the drug which otherwise would accumulate in toxic concentration in nervous tissues and distribute it amongst the tissues of the body not specifically affected by the drug. When the fluid is excreted by the kidneys the urine contains barbiturates in higher concentration than exists in any of the tissues of the body.'

Koppanyi and Fazekas,[11] on the basis of these experiments and Koppanyi and Linegar's[15] studies on cross-circulation, suggested dialysis with an artificial kidney for the treatment of acute barbiturate poisoning. Several reports from the Department of Medicine of Georgetown University indicate that the artificial kidney is indeed a life-saving measure in barbiturate poisoning, particularly in cases of phenobarbital intoxications.[16-18] At the present time, the artificial kidney is a rather cumbersome and expensive apparatus, but in the near future Professor Kolff's simple and inexpensive design will become available for general clinical use.

* Through the courtesy of Prof. F. H. Shaw of the University of Melbourne, the author was the first, at least in the United States, to obtain a large sample of beta beta methyl ethyl glutarimide, which was recommended for the treatment of acute barbiturate poisoning.

The experimental data obtained in my laboratory indicated that this drug is an effective agent in acute barbiturate poisoning, but not more so than picrotoxin or leptazol. It is interesting to note that a newer hypnotic, doriden, which is also a glutarimide, is apparently more effectively antagonized by megimide than are barbiturates. However, even in this case, the antagonism is a physiological one that is due to the central stimulating and/or convulsant properties of megimide and not because of a specific antidotal effect like nalophine versus morphine or para-amino-benzoic acid versus sulphonamides. The barbiturate or doriden-intoxicated animal or patient may awaken despite the high blood levels of soporific drugs. If we keep it in mind that megimide is a convulsant, and look for early symptoms of central excitation in patients depressed by barbiturates or doriden, beta beta methyl glutarimide may be recommended in the treatment of clinical poisoning,

Summary

The symptoms of acute barbiturate intoxication are essentially those of surgical anesthesia. Differential diagnosis, therefore, must depend on the history (which is often misleading), and chemical identification of the offending agent.

Chemical determination of barbiturates in the blood and tissues is described.

Symptomatic and analeptic therapy in barbiturate poisoning are described, along with treatment of this intoxication by massive infusion or the artificial kidney.

Table 2: Clinical and Toxic Doses and Comatose Blood Levels of the More Important Barbiturates

Compound	*Usual Dose (Grams)*	*Severe Intoxication but Generally with Recovery (Grams)*	*Generally Fatal (Grams)*	*Comatose Blood Levels (Mg./100 c.c.)*
Alurate ..	0·3	5—10	6—10	5—10
Amytal ..	0·1—0·3	1·5—2	2—3	2—4
Barbital ..	0·5	3—>10	5—>20	10—20
Dial	0·1—0·3	2—2·5	>2·5	2—4
Pentobarbital ..	0·1—0·2	>0·5	>1	1·5
Pernocton ..	0·2—0·3	0·5—1·0	>1	1—2
Phanodorn ..	0·1—0·3	1·2	>10	5—10
Phenobarbital ..	0·1—0·2	4—5	5—9	7—12·5
Seconal ..	0·1	>0·5	>1	1·2—1·5

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Sex In Negro Skeletons

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Techniques for accurately determining the sex of a skeleton are obviously of interest to persons concerned with the identification of human remains. This paper presents several precise techniques which are of special value for determining the sex of Negro skeletal material, and of general value for specimens from other racial groups.

Materials, Methods and Results

Through the courtesy of Dr. Mildred Trotter and with the support of funds from the Wenner-Gren Foundation for Anthropological Research, a sample from the skeletal material of the Terry Collection at Washington University in St. Louis was used for this study. Adult males (N = 99) and adult females (N = 101) of known sex but picked at random from the larger total series were measured for the following:

A. Femur length (bicondylar).
B. Femur head diameter.
C. Humerus length.
D. Epicondylar width of the humerus.
E. Clavicle length.
F. Ischium length.
G. Pubis length.
H. Sternum width.

These data were handled so that values necessary for usual statistical description and for discriminant function analysis could be done. Various indices and sorting combinations were tried in the hope that by some technique it would be possible to sort with 100% accuracy. This paper does not present all of the techniques that were tried, as many were without value, but describes those that have the highest practical value.

Table 1 gives the means and their errors and the critical ratios ('t' values) from comparisons of male-female values for each measurement.[1] From this it may be seen that while the sexes are different for each measurement at a highly significant level, the difference is not the same for each. We may conclude, then, that sexual dimorphism differs by measurement and that some measurements are more useful for discriminating between the sexes than others.

1. The total sample of 99 male and 101 female was not used in calculating the statistical values as 2 individuals, one of each sex, could not be measured for the epicondylar width of the humerus. Inasmuch as cross-products are needed in the discriminant analysis, these 2 were omitted. As a consequence, the total sample, while shown in Figs. 1 and 2, is reduced to 98 males and 100 females in Tables 1 and 2. These 2 individuals were not in the intermediate range.

Table 1: Statistical Values Derived from Various Measurements taken from Male and Female American Negro Skeletons of Known Sex

Measurement	*Sex*	*Number*	*Mean (mm.)*	*Standard Deviation*	*Standard Error of Mean*	*Critical Ratio ('t')*
A. Femur length ..	M	98	477·34	28·37	2·866	10·13
	F	100	439·10	24·55	2·456	
B. Femur head diameter	M	98	47·17	2·75	0·278	16·17
	F	100	41·52	2·12	0·212	
C. Humerus length ..	M	98	338·98	18·55	1·874	12·51
	F	100	305·89	18·66	1·866	
D. Epicondylar width of humerus ..	M	98	63·89	3·59	0·363	14·50
	F	100	56·76	3·32	0·332	
E. Clavicle length ..	M	98	158·24	10·06	1·158	13·90
	F	100	140·28	7·99	0·800	
F. Ischium length ..	M	98	90·55	5·06	0·511	13·52
	F	100	81·80	4·00	0·400	
G. Pubis length ..	M	98	74·14	5·11	0·516	6·39
	F	100	78·21	4·34	0·434	
H. Sternum length ..	M	99	36·15	3·91	0·393	9·66
	F	98	30·96	3·63	0·367	

As the sample reported here is composed of American Negroes, the applicability of these data to other Negro populations is of interest. In Fig. 1 the histogram array of I/P (ischium : pubis) index[2] from this series is given together with Washburn's (1949) series of 152 Bantu skeletons of known sex. The upper range for males is the same for both, but the lower range for females is 85 for American Negro and 87 for Bantu. This is not a great difference and may be a consequence of differences in sample

2. The following is a quotation from Washburn (1948) describing the method used in taking the measurements necessary for computing the I/P index: "The technique of measurement used was that described by Schultz (1930). Length of ischium and pubis were measured from the point at which they meet in the acetabulum. This point can be identified approximately in the adult because:
(i) Frequently, there is an irregularity there, both in the acetabulum and inside the pelvis;
(ii) There is a change in thickness which may be seen by holding the bone up to the light;
(iii) Often there is a notch in the border of the articular surface in the acetabulum.
In measuring the pubis care should be taken to hold the calipers parallel to the long axis of the bone."

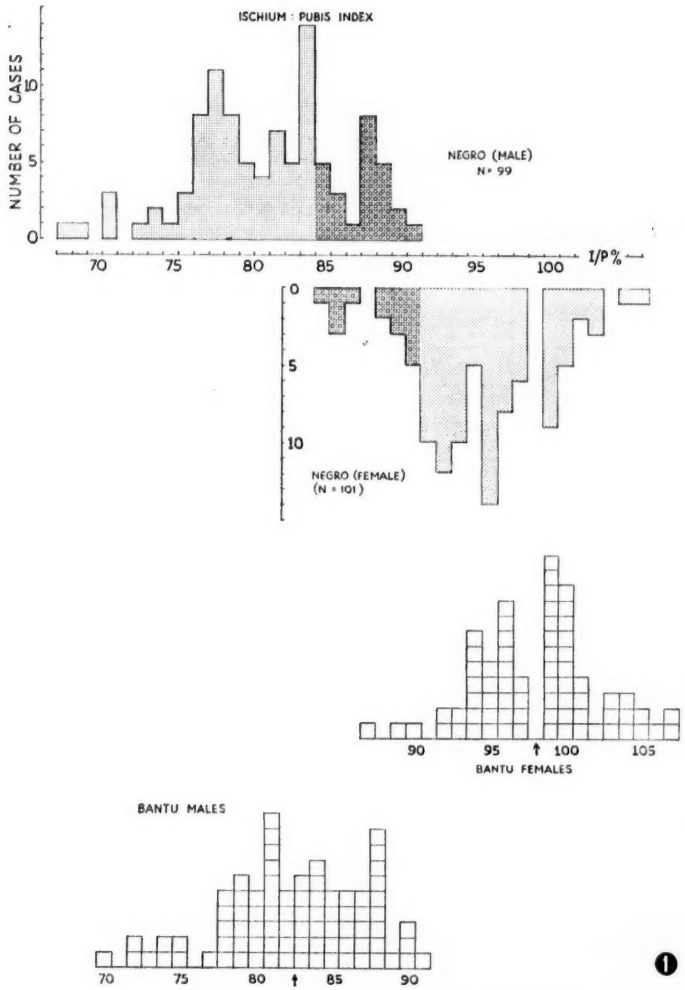

Fig. 1. Histograms giving the I/P (ischium : pubis) index distribution by sex for 200 American Negroes of this study compared with 152 Bantu (taken from Washburn, 1949).

In the upper half of the figure the dark shading is for those individuals who lie in "overlap" area between 85·0 and 92·0. These 40 individuals are the ones who are shown distributed in the arrays of Fig. 2.

size. The value of 89·0 for the American series from the Terry collection separates all but 5 females and 8 males correctly (93·5%). The very same value in Washburn's Bantu series separates all but 5 males and 1 female

correctly, or at a level of 6 out of 152 (96·1% level). Washburn (1948) reporting on the I/P index for known sex Negro skeletons from the Hamann Museum collection at Western Reserve University in Cleveland shows that again a limit value of 89·0 is best for Negro material (4 females only out of 100 skeletons not properly sexed, or the 96% accuracy level). In all probability, from what we see of this comparison between American Negro and Bantu I/P index, other discriminating measures or techniques derived from the American data will be useful for other related populations.

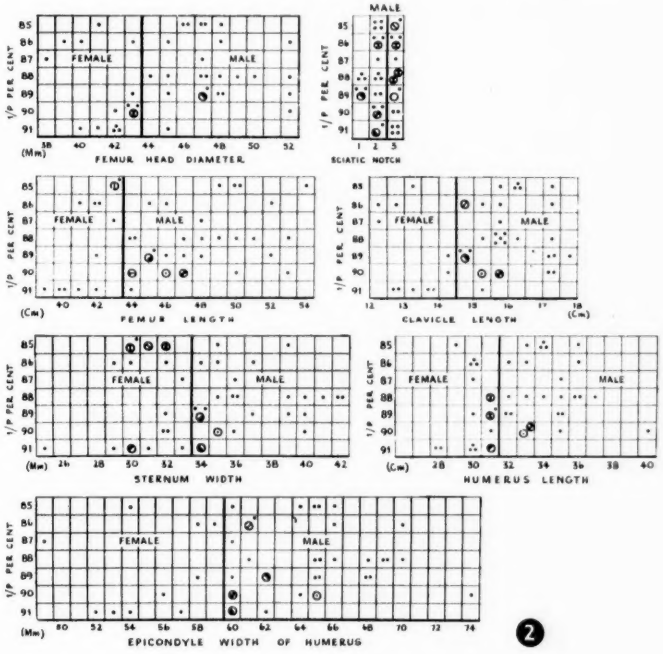

Fig. 2. Distribution of 40 individuals shown in the dark-shaded area in the upper group of Fig. 1, who have an I/P index between 85·0 and 92·0. These persons are shown here distributed according to their measurements for different bone dimensions and for sciatic notch opening.

The small dots indicate persons who would be correctly sexed by the dividing point indicated by the heavy vertical line. The larger symbols are for those not correctly sexed. The large symbols are assigned to individuals so that those not correctly sexed for more than one measurement are shown. All those who appear out of the correct range only once are shown with the symbol ⊗.

Fig. 2 shows the result of an analysis done to find the sorting value other measurements may have for those specimens not correctly sorted by the I/P (ischium : pubis) index alone. In our sample 40 of 200 individuals are not correctly sorted if all specimens with an index less than 85 are considered

to be male and all over 91 to be female. Fig. 2 then presents six[3] other measurements for these uncertain specimens scaled according to size. Also included are the observations on sciatic notch opening and the I/P index. In addition, a special symbol is assigned to those individuals who are not sexed by any one measure so that they may be followed to see how they fare in each array. This is done so that the individual specimen and not disembodied measurements can be followed in the analysis. Where the same symbol appears repeatedly it is apparent that for several measurements that person is out of the expected size range for his sex. This arrangement gives an idea of the way some of the difficult to sex individuals vary or the different measurements.

The best single discriminating measurement, next to the I/P index, is femur head diameter. Only 2 persons not sexed by ischium : pubis index are not sorted if all under 44 mm. diameter are considered to be female and those 44 mm. and above to be male. This method cannot be carried one step further, however. Even though the symbols of the 2 individuals not sorted by femur head diameter do not appear in the femur length or humerus length arrays, it does not follow that those 2 deviant individuals could be picked out of the sample if sex were unknown. Despite this, it is quite accurate to conclude that *in this sample* we can sex with 99% accuracy if we first sort for I/P index (and call all individuals male if under 85 and all female who are over 91) and next sort those remaining (calling all under 44 mm. for femur head female and all others male). The resulting male-female groups would have, for this sample, one individual per 100 incorrectly sexed. The method would very probably give less than 5% error in any other sample drawn from Negro populations. The precise level, however, is not known from this approach as it does not derive or generalize an estimate of probability from sample variability.

From Table 1 it is apparent that femur head diameter is the best single measurement for discriminating the sexes in this series. For this reason, a rough attempt was made to see how useful an index including pubis, ischium and femur head would be. Inasmuch as acetabulum size is highly correlated with femur head size, but because it is not presently feasible to remeasure the series, another method was used. Pubis length minus half of femur head diameter was divided by ischium length plus femur head diameter to get an index value. In this series, when this index is under 0·500 the specimen is male and when it is over this value it is correctly sexed as female in 96·5% of the cases. It is therefore indicated that an improved index could be gotten by taking the shortest distance from the rim of the acetabulum to the superior point on the pubic symphysis and dividing it by the distance from the ischium point to the far rim of the acetabulum.

3. The opening of the sciatic notch was observed and rated on a scale of 1—5, with 1 being the extreme, restricted opening for males and 5 the most open condition.

The limit value of 0·500 would undoubtedly not be the same, but this index would be a highly efficient simple index for sexing. It has the advantage over the regular I/P index in that it maximizes the values that the pubis and ischium *plus* the femur head have for sex discrimination. In addition, the landmarks are well defined and the measurements easily taken.

Table 2: Discriminant Analysis Results giving Multipliers and Limit Values for Various Combinations of Measurements

Measurements and Their Multipliers for Use in Sex Discriminations

	7 × 7	6 × 6	4 × 4	3 × 3
A. Femur length	0·07	1·00	1·00	1·98
B. Femur head diameter	58·14	31·40	16·53	
F. Ischium	16·25	11·12	6·10	1·00
G. Pubis length	—63·64	—34·47	—13·80	—1·39
C. Humerus length	2·68	2·45		
D. Epicondylar width of humerus ..	27·68	16·24		
E. Clavicle length	16·09			

Discriminating Values

Measurement Combination	*Value Separating the Sexes (Females less than the Value)*	*Probability of Error in Classification (%)*	*Range Within which 99% will Fall*
7 × 7	4099	1·5	2664–5533
6 × 6	1953	2·5	1205–2701
4 × 4	665	3·1	367–963
3 × 3	68	6·5	50–86

Table 2 gives the values which are most efficient for sexing when all the measurements or certain combinations of them are used together. These were derived by discriminant analysis[4] and have been explained in terms of theory and method of derivation in considerable detail in another paper (Thieme and Schull, in the press). This type of analysis has an advantage over the previously mentioned methods in that a probability statement is derived which is applicable to all series from the same population and is not merely an expression of the events in the single series. For instance, while the I/P index limit of 89·0 sorts all but 13 of our series correctly, there is no assurance that this level of results would be the same for any other group. In discriminant analysis, using the variability expressed within each sex and between each sex, a general statement of probability can be obtained.

In practice the results in Table 2 are quite simple to use and can be applied in the following fashion. As shown in the Table, 3, 4, 6 or 7 measurements

4. The discriminant function analysis was done by Dr. W. J. Schull, Heredity Clinic, University of Michigan. The author acknowledges this service with gratitude.

are taken. The appropriate multipliers are used and the totals of these products (measurement value in millimeters times the appropriate multiplier) are obtained. If the total is under the limit value given for the number of measurements used, it is a female specimen and if over, it is male.

For example, when applied to an actual case which cannot be sexed by I/P index or femur head size (female, I/P index 86·9) with 4 measurements as follows:

1. Femur length = 429 mm.
2. Femur head diameter = 44 mm.
3. Ischium length = 84 mm.
4. Publis length = 73 mm.

The multipliers from Table 2 give:

1.	1·00 × 429 =	429·00
2.	16·53 × 44 =	727·32
3.	6·10 × 84 =	512·40
4.	—13·80 × 73 =	—1007·40
	Total =	661·32

As the limiting value is 665 and all under this are female, this skeleton is classified correctly as female.

The more measurements that are used reduces the likelihood of error. If for a single specimen the total of the calculations is quite distant from the limit value, sex can be assigned with great certainty. If a series of skeletons are being sexed, the probability of correct assignment is given in the table for the number of measurements used.

Discussion

Sexual dimorphism in Man is probably quite constant and the degree of difference between the sexes within any human population is not significantly variable (Hrdlicka, 1947; Schultz, 1937). The absolute size of human beings is, in contrast, quite variable and racial or population differences may be quite significant. In problems of sexing, this leads us to conclude that the criteria applicable to one race may be useful for any other if the appropriate limit values discriminating between the sexes can be established. To put a question in practical terms: how can the data from the American Negro be used (and with what level of accuracy) on other populations of different racial extraction? Sexing unknown specimens with accuracy depends upon 2 bits of information. The first is the degree of sex difference within the population. As mentioned above, we can probably operate effectively on the assumption that this is a constant value for all human varieties. The second bit of necessary information is the absolute size of the limit value that most efficiently divides the variability curves of males and females in a population. This limit value is a function of the absolute size of the particular variety of mankind in question and can be different for each population. In this paper we have presented data to show that for American

Negro and Bantu there is probably great similarity in the bones that go into making the I/P index such that the limit values for each population are the same. Probably any Negro population will be quite close to these same values. Unfortunately we do not have data from known sexed skeletons for many other populations which would allow us to evaluate the situation for other varieties of Man. Despite this situation, it is proper to point out that, if the mean values by sex for the measurements used here were known for any other race, an appropriate limit which would be applicable to an individual specimen can be calculated quite easily. The appropriate multipliers of the discriminant function analysis results given in Table 2 can be applied to the mean values and half the difference between the results for males and for females will give the proper limit value. The discriminant analysis assumes normality of distribution so the limit value can be obtained if the means for each sex are known.

The discussion so far has dealt with the problem of the applicability of findings from one population to other groups, but inasmuch as most problems of identification deal with individual specimens some mention of this should be made. The statement of the probability of correct sexing, which is an inherent property of a particular method, is subject to modification when applied to individual cases from a population. For instance, if the I/P index of one individual is 88·0 and for another it is 78·0, there is not equal likelihood that they are both male. In the first case it is quite dubious and in the second, with 78·0, it is very certain. In fact, the certainty is a function of the distance of the individual value from the appropriate group limit value. In the case of the specimen with a value of 78·0 there is less than a 1:1,000 chance that it is not male. With an understanding of the nature of variability for sex in each of the methods given in this paper, an experienced person can decide how he will go about sexing a specimen. For instance, whether the 7 measurement discriminant value must be resorted to is decided on the basis of the place of the specimen in the known pattern of sex variability. In a number of cases the single I/P index will be all that is necessary for sexing with satisfactory degrees of certainty.

Some final remarks about the desirability of using measurement for sexing are in order. Traditional methods for the assignment of sex on the pattern of skeletal morphology do not have an explicit basis. An experienced investigator can attain very high degrees of accuracy with such methods, but there is usually very little chance for him to check his estimations against known material. The result is that while the accuracy may be high (or it may not be) there is no explicit statement of probability that is inherent in the method. We are forced to evaluate the expert and cannot evaluate his methods except to note his experience. In contrast, measurement methods admit the possibility of deriving an accurate statement of probability of correct sexing which is independent of the person and inherent

in the method. The appeal of a measurement method is not alone the simplicity, but also the degree that science replaces art. There are cases where fragmentary evidence and unusual circumstances demand the services of an expert who must almost intuitively arrive at conclusions, but this does not mean that such an approach is satisfactory when it is unnecessary. The introduction of precise measurement methods does not rule against the need for the expert, but rather adds variety to his methods and demonstrable certainty to his conclusions.

Conclusions

1. Techniques especially applicable to sexing Negro skeletons have been derived from a sample of adult specimens of known sex.

2. With the ischium : pubis (I/P) index, all specimens under 89·0 are male and all over are female, with the chance of error in assignment being less than 10%.

3. (*a*) All specimens with an I/P index of less than 85·0 may be considered to be male and all female which are over 92·0.

(*b*) For those between 85·0 and 92·0, all with a femur head diameter less than 44 mm. are female and all over are male. If 'a' and then 'b' are carried out in sequence, there is about a 95%, or better, chance that sex will be correctly assigned. Other measurements and limit values may be substituted for femur head diameter in step 'b' as indicated in Fig. 2.

4. Discriminant analysis was used to derive limit values and appropriate multipliers to be used with various combinations of measurements (Table 2). The maximum discrimination can be obtained from 7 measurements. With this method the probability of error can be reduced to 1·5%, giving 98·5% expectation of accuracy in known material and double this, or 3% error, when the material is unknown.

5. The foregoing precise sexing techniques are derived from an American Negro series and are most applicable to specimens or series derived from that population. Comparisons with Washburn's (1949) Bantu material indicates that they may be equally useful for other Negro populations. Their applicability to more distantly related populations is a function of the degree of phenotypic difference. Due to the high stability in expression of sex dimorphism in Man, the probability of error remains unchanged if appropriate limits for other groups are used.

6. Limit values for other populations may be derived if mean values for measurements from known sex skeletons are available. In such cases, the discriminant function analysis values of Table 2 are applied to the means for each sex. The limit value for the other population is then half the difference between the totals derived for each sex.

7. For sexing individual specimens the techniques proposed are especially efficient if the derived values are quite different from the limit values separat-

ing the sexes. Absolute certainty in sexing can be achieved in cases where the difference is great.

8. It is recommended that sexing be done by using certain explicit methods so that estimates of error, which are inherent in each particular method, can be stated. Using traditional methods whereby various hallmarks of sex are evaluated in the light of morphological form gives no clear basis for estimating the likelihood of error even when done by an experienced investigator.

9. A new index, more accurate and with better defined landmarks than the I/P index, is proposed. It uses the length from the nearest border of the acetabulum to the symphysis pubis, divided by the length from the ischium to the farthest border of the acetabulum. The index value dividing the sexes is approximately 0·5.

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Medico-Biological Interpretation in Cases of Sexual Delinquency

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The function of medico-biological evidence in cases of sexual crimes is very varied. Interest should not be confined to the demonstration of microscopic evidences of spermatozoa. Our experience on the large and varied material of a German State C.I.D. has shown that answers can be found to questions of much wider importance. The combination of medical opinion with a general biological outlook can be decisive in elucidating a criminal act. We present in the following descriptions a few examples of various types of case. The methods employed which have proved useful will be given at the end of this paper.

Sperm Findings and the Time of the Act

Case 1

Policeman Helmut E. had to take Herma B., a 17-year-old female, from the town N. to the prison in A., a distance of about 200 kilometres. According to subsequent evidence, the policeman behaved improperly during the train journey, when he offered the girl cigarettes and chocolates. They had to change trains in Munich. He did not board the next train but took his prisoner to a cinema. Because of this delay, their destination was not reached that evening and he rented a double room in an hotel at H., where he spent the night with the girl. The following morning she refused to get up and follow him to the prison. After protracted negotiations he was compelled to approach the nearest police station for assistance. The girl now alleged that she had been raped by the policeman during the night. Policeman E. (already incriminated by his incorrect behaviour during the journey and the over-night stay at the hotel) not only denied having employed force or persuasion, but also declared that he had had no relations whatsoever with the girl. Further evidence was considered necessary because of the bad reputation of the accused girl.

We examined stains found on the sheet of the bed in the hotel room.

These were somewhat reddish and did not fluoresce. Microscopic examination showed ample spermatozoa and also the presence of glycogen-containing epithelial cells together with traces of blood, which explained the absence of fluorescence.

The phosphatase reaction was distinctly positive (*vide infra*, 'Methods'). The determination of the age of the stains according to Weinig (*vide infra*, 'Methods') showed no migration of ions. It could be said on the strength of these findings that the fresh stains were caused by a mixture of sperm and vaginal secretion, probably mixed with some menstrual blood. These traces could only have one source, viz. reflux of the ejaculate from the vagina of a menstruating woman.

The results of the examination appeared to have proved beyond doubt that intercourse had taken place with the girl. The accused insisted that he had had no intercourse with her. He adduced evidence in court that the girl had had intercourse with someone shortly before her arrest, about 20 hours before this particular night, and alleged that this could account for the finding of spermatozoa. This statement was refuted because the findings virtually excluded the possibility of sperm from an intercourse 20 hours before the said night. The reasons were:

1. *Massive numbers of spermatozoa were found.* There are, as a rule, no spermatozoa present in the vagina of a living woman 20 hours *post coitum*. No accurate investigation of this problem has been reported, but our experience has revealed that after such a long period one could, at best, find only a few isolated spermatozoa.

2. *The positive phosphatase reaction.* According to Fisher, this reaction will not remain positive in the vaginal secretion of a living woman for longer than 12 hours after the deposition of the ejaculate.

3. *The elimination of sperm from the vagina* was speeded up by the increased secretion due by the end of menstruation (corroborated by the evidence of the girl).

4. *Comparison of the blood group of the accused man with that of the sperm* could not be carried out in this case, as the sheet had been returned to the owner and had been washed.

The accused objected to the expert evidence that the stains had not been present on the sheet before he had rented the room. Since the room had not been occupied for 14 days before the act and the hotel keeper could prove that the bed linen had been changed, this last defence was refuted.

Case 2

A 32-year-old shy farmer, without any signs of psychological peculiarities, had had no opportunity of meeting female company. He therefore decided to use violence in order to have intercourse with a woman who used to

pick berries in the woods. After several unsuccessful attempts, he ambushed her and hit her over the head with a garden hoe. He completely smashed the head of this 42-year-old woman and performed the sexual act on the dead body, which was found 16 days later. It was in a state of extensive decomposition because of the summer heat. The skin showed dark green discoloration and was partially separated from the underlying tissues. The body cavities were distended by gases and there was extensive damage by mites. Microscopic detection of sperm was nevertheless easy, but the chemical tests for sperm were negative.

This case showed that, in contrast with the case of a living woman, sperm remains detectable for days or weeks in the vagina of the body. By incubating spermatozoa we have obtained experimental proof of their resistance to putrefaction.

Evidences of Zoophilia

Case 3

A married foreman was reported 3 times in 9 months for sexual abuse of cattle. His arrest was made possible through the systematic analysis of traces. Macroscopic examination showed on the jacket and trousers of the accused hairs which were identified by microscopic examination of their cuticle and medulla, as originating from cattle. The hairs lay loose on the front and on the sleeves of the jacket, but masses of short cattle hair were found about the fly of the trousers, partly caught under the buttons. They were especially numerous near the lower end, on the inner surface of the fly, permitting the conclusion that they could only have got there through intimate contact with the skin of cattle while the fly was open. In addition, glycogen-containing epithelial cells from cattle vagina and human spermatozoa were found on the edges of the fly. (Protein precipitin tests for cattle and Man, according to Uhlenhuth, were positive). There were residues of cow dung on the shoes of the accused. Correlating all these findings, expert opinion could be given, with a probability verging on certainty, that the wearer of the garment had had sexual intercourse with cattle.

Case 4

Dead hens were frequently found in the fowl runs of 2 neighbouring farms. It had to be decided whether a hen handed over to the expert had been sexually abused by an unknown offender.

The post-mortem examination of the animal gave the following results:

The cloacal orifice was smeared with blood and the surrounding feathers were bent. The cloacal sphincter was undamaged, but the anterior wall of the cloaca, about 3 cm. above the sphincter, was lacerated (by blunt force)

and communicated with the abdominal cavity. The abdominal cavity contained approximately 20 c.c. dirty fluid blood and several blood clots, and the intestines were partly covered with fresh fibrinous deposits. The root of the mesentery, up to the diaphragm, was suffused; the connective tissue, especially round the left Mullerian duct, was permeated with blood. Spermatozoa could not be found among the innumerable leucocytes in the fluid present in the abdominal cavity, but the protein precipitin reaction against anti-human serum gave an unequivocal, positive result. The phosphatase reaction was also weakly positive.

The hen had died from internal haemorrhage and incipient peritonitis caused by rupture of the wall of the cloaca, due to a blunt force.

The presence of human protein, together with the positive phosphatase reaction, indicated with the greatest probability that there was human sperm in the abdominal cavity of the animal. No other explanation could be found than that the injury to the animal was caused by the introduction of the penis of an unknown offender.

Following another occurrence of the same kind, a man of the neighbourhood was arrested. He had been seen drunk, during the night, near the place of the act. His clothing was sent to us and the examination yielded the following results:

Numerous circular or irregular greyish brown dried stains were noticed on the trousers in the region of the lower abdomen and the thighs, and especially round the fly. Microscopic examination of the stains revealed mostly traces of earth grossly mixed with decaying organic matter. The presence of faeces was particularly conspicuous.

The identification of grain particles and of the epidermis of grain longitudinal cells of the walls of maize fruit and of potato peel, all pointed with great probability to fowl faeces.

Especially significant was the presence of minute tertiary fibre fragments of hen feathers. Examination with a magnifying glass revealed tiny whitish to brownish feather fragments of which one was attached to the outer, and two to the inner surface of the fly. On microscopic examination these were identified as fowl feathers.

Finally, there were dry, whitish stains on the external as well as the internal surfaces of the fly near its edges. Microscopic examination revealed that they consisted mainly of epithelial cells, partly containing glycogen, and they were mixed with isolated traces of faeces and very small chicken feathers. Spermatozoa could not be detected. Uhlenhuth's protein precipitin test with anti-chicken serum gave a distinctly positive reaction, whilst the tests against anti-human and anti-sera prepared against other animal proteins gave consistently negative results.

There were also on the underpants several black, greenish-brown stains, again on the outer as well as on the inner surfaces of the fly. Microscopic

examination showed that they were identical with the stains found on the trousers, and consisted of faecal particles and epithelial cells; spermatozoa were not found. We found, however, a highly characteristic element of the fowl cloaca, eggs of the fowl parasite *Distomum pellucidum*.

The protein precipitin test against human and chicken anti-serum yielded weakly positive results, whereas all control tests were negative. The phosphatase reaction was also weakly positive.

The following conclusions were drawn:

Traces were found on the shoes and clothing of the suspect which on microscopic examination proved to have originated from the surface dirt of a fowl run. These traces, together with fine feather particles and cloacal secretion of the hen were so distributed on the outer and inner surfaces of the fly that they permitted the conclusion of an unnatural sexual act with hens. There was only one explanation of the origin of the stains on the trousers and underpants, viz. that the fly and its vicinity came in contact with the external cloacal region of a hen or hens, which smeared the surface dirt of the run with their trampling feet onto the trousers, and that cloacal secretion and faecal particles were deposited on the inner surface of the trousers and underpants on withdrawal of the penis.

Differentiation Between Blood Traces Originating from the Genital Organs and Analysis of Evidences from the Place of the Criminal Act

Case 5

J. B., married, 29 years old, was accused of having dragged M. St. (a girl of 19 years) from the street and, by the use of threats, of having completely undressed and raped her.

Medical examination proved that St. was on this occasion robbed of her virginity.

Examination yielded the following results.

1. Head hairs found on the collar of the accused's jacket were identical with those of the wronged girl and, on the basis of their micro-anatomical details, could be recognized as not being hairs of the accused.

2. There were traces of vaginal secretion and blood on the fly of the trousers, the edge of the shirt and on the underpants of the accused belonging to the same blood group as the blood of the girl and different from the blood of B's wife.

A fibrinolytic potential characteristic of menstrual blood in contrast to that of blood originating from defloration or in abortion could not be detected. (*vide infra*, 'Methods').

The microscopic examination of the blood traces showed well preserved red cells, no endometrial cells of the uterus and few bacteria, findings which are not consistent with the features of menstrual blood.

3. The girl's coat on which the accused forced her to lie showed traces of fresh sperm belonging to the same group as the blood group of the accused.

4. The ground at the scene of the assault was of turfy earth, rich in humified fragments of higher plants. It also contained certain distinct diatomaceous elements. The same elements were found on the coat of the girl and on the tips of the accused's shoes. The accused was alleged to have soiled his shoes and clothes with earth in front of his lodgings, in the street, but the ground there had an entirely different structure consisting mainly of quartz crystals free from plant fragments and diatomaceous elements.

Each finding by itself had little weight, but their sum together with the evidence of the girl was enough to lead to the arrest of the accused.

Methods

1. Phosphatase Reaction (a) Reagents (Author's Modification)

Solution I (*Buffer*)	Sodium chloride	23 g.
	Sodium acetate 3 H2O	2 g.
	Glacial acetic acid	0.5 c.c.
	Distilled water	90.0 c.c.
Solution II	Dianisyltetrazonium chloride	30 mg.
	Ca-α-naphthylphosphate	50 mg.
	Sodium laurylsulphonate 10% aqueous solution	1 c.c.

Filter

Solutions I and II are mixed to give the reagent. The mixture is stable in the refrigerator for a long time but it is advisable to prepare it fresh once monthly. Solution I is stable.

(*b*) Procedure

The part considered suspect on the basis of the preliminary examination is cut out as well as a piece of the same material which is unstained and serves as control.

Both pieces are placed, each in a suitable porcelain dish, and the reagent is added through a filter. If the stain contains sperm, the acid phosphatase liberates α-naphthol the fromester combination and this produces with the diazonium salt a blue-purple azo dye. The stain shows the colour within a few minutes, whereas the control remains unchanged. The reaction is positive with sperm even if it is 1-3 years old. With the reagent ready, the test only takes a few seconds and can be read in 5-10 minutes.

The value of this phosphatase reaction as it had been elaborated by the author cannot as yet be finally assessed despite numerous experiments and much practical experience. Fisher and Gradwohl consider the Walker test as conclusive. The unequivocally positive dianisyltetrazonium chloride

reaction has always coincided in our material with the microscopic detection of sperm and we never have found sperms microscopically when the reaction was negative. Our experience has given us the impression that the reaction is conclusive if only the blue-purple colour is considered as a positive reaction. Yellow or brownish colouration may appear with stains of urine, vaginal secretion and with certain foodstuffs.

2. Determination of the Age of Sperm (Weinig)

The aim of the test is to make visible the migration into adjacent areas of chloride and sulphate ions which takes place in the case of blood and sperm stains and also with ink writing.

A wedge-shaped piece is cut out from the area of the stain, the point of the wedge reaching into the actual stain. It is subjected to the following chemical treatment in order to determine the chloride pattern and, if necessary, another piece is examined for the sulphate pattern.

(*a*) *Chloride Reaction:* (1) The material is placed for 3 minutes in a solution of silver nitrate, using a 1:100 solution for stains on paper and a 1:1000 solution for stains on textiles. Before placing the specimen in the silver solution, add to the solution 5-6 drops of concentrated nitric acid.

(2) The specimen is soaked 3 times, 2 minutes each time, in fresh 1% nitric acid (avoid bright light).

(3) It is washed briefly in distilled water.

(4) Then it is placed for 3 minutes in a mixture of 1 part 35% formaldehyde and 9 parts 2% sodium hydroxide.

(5) It is washed in distilled water.

(6) Finally it is dried on filter paper.

(*b*) *Sulphate Reaction:* (1) Place the wedge-shaped piece for 3-4 minutes in 4% lead nitrate solution.

(2) Wash 3 times, 2 minutes each time, in saturated lead sulphate solution.

(3) Rinse with distilled water.

(4) Place specimen for 3 minutes in a mixture of 2·5% sodium sulphide and 2% sodium hydroxide.

(5) Rinse in distilled water.

(6) Dry on filter paper.

The breadth of the chloride- and sulphate-containing areas becomes particularly distinct if the piece is replaced in position from where it was cut out.

3. Determination of Fibrinolysis for the Detection of Menstrual Blood

The differentiation of menstrual blood from blood which originates from the vagina but is not menstrual became possible for the first time in 1952 when the author, basing himself upon physiological considerations, tried

to utilize the assumed fibrinolytic properties of menstrual blood for forensic purposes. As is known, menstrual blood is usually liquid and does not clot. The reason is that the blood probably clots at first in the uterus and becomes liquefied afterwards. Irrespective of the explanation, it was proved that menstrual blood contains an active substance capable of lysing fibrin. This fibrinolysin is also present and can be detected in an extract of dried menstrual blood. For practical purposes the following method has been devised:

The saline extract of the stain is made up to 2 c.c. and is distributed in 2 tubes, 1 c.c. each. Fresh human fibrin is added to the one tube and both tubes are incubated at 37°C for 24 hours. The degree of fibrinolysis is determined from the difference in nitrogen content in the control and the fibrin tube, the nitrogen being determined with the semi-micro-Kjeldahl method. Menstrual blood shows considerable lysis, whereas venous blood collected during operations on the female genitals or from abortion bleeding, has no or insignificant lysing power. The more concentrated the extract, the higher the lytic values obtained. It is therefore desirable to keep the fluid volume used for the extraction of the traces low. The technique is not difficult but utmost accuracy is required.

Our experience has shown that menstrual blood very often shows distinct fibrinolysis but this may also be absent. On the other hand, genital blood traces that are not menstrual have never given a positive fibrinolytic result.

According to our results, a lytic value above 10 mg. per 100 c.c. may be considered as a positive reaction for menstrual blood.

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Notes and News

When is a Drug not a Drug?

Last week, in a paragraph on *Armstrong v. Clark* (reported in *The Times*, 31st January, 1957), we described the Divisional Court's decision as one of gravity, and it does appear to raise a number of awkward questions. Is insulin a drug within the meaning of s. 15 (1) of the Road Traffic Act, 1930, and *semble* also s. 9 of the Road Traffic Act, 1956? No, said the Prestbury Justices, applying to the word 'drug' what must now be described as an academic construction. But the Divisional Court sent the case back to the magistrates and the Lord Chief Justice said in no uncertain terms that he and his brethren had no doubt whatsoever, that insulin was a drug: 'drug,' said the Lord Chief Justice means 'medicine or a medicament—something given to cure, alleviate or assist an ailing body,' and went on to say that insulin clearly comes within this definition. It is a substance given to enable the diabetic to change the glucose circulating in his blood into glycogen which can be stored in the liver and muscles until required for the release of energy; if there is insufficient insulin being produced by the pancreatic gland the glucose derived from the carbohydrate food reaches dangerous levels in the blood stream and eventually this may cause coma and even death. On the other hand, too much insulin, relative to the amount of glucose circulating in the blood, will cause the level of glucose in the blood stream to fall below that which is necessary to provide a proper environment for the operation of the cells in the brain, and this causes first a lack of co-ordination and control, then coma and eventually death.

When it's a Suet Pudding?

It is clear that if a man has a dose of insulin and then drives a car he may run the risk of his brain being affected by the injection sufficiently to make his driving unsafe, and he should, therefore, in the public interest, take the precaution of eating the appropriate amount of carbohydrate food to avoid such a dangerous situation. If he allows himself to be affected by the injection then he comes within the relevant sections of the Road Traffic Acts. But what if a diabetic takes a large carbohydrate meal and fails to have an insulin injection? If he goes into a diabetic coma while driving, is he 'under the influence of a drug'? Are mashed potatoes, suet puddings and ice-cream 'drugs'? Or are they capable of becoming 'drugs' in certain circumstances?

This, with the greatest respect, is the weakness of the Lord Chief Justice's definition. It may be that no satisfactory definition can be found and indeed

that none is desirable. It is obvious from his direction to the justices that the Lord Chief Justice feels that anyone who drives while in a condition of health which renders him subject to comas should come within this section; but to include a diabetic suffering from coma due to excessive indulgence in carbohydrate is, it is submitted, straining the meaning of the words of the Act: can any possible construction of the word 'drug' include ordinary foods which are only dangerous in certain circumstances and to certain individuals?

The word 'drug' has to most people certain overtones of vice; 'taking drugs' is generally felt to be a bad thing, and it is interesting to notice the way the advertisers of patent medicines do everything possible to assure the public that no drugs go into their products. 'Contains no drugs!' scream the advertisements, 'Nature's way is best!' and so on. Then, if they are to be believed, according to the Lord Chief Justice their product can be of little value, since it contains nothing to 'cure, alleviate or assist an ailing body.' To push the matter to the *reductio ad absurdum*, a pastrycook might advise: 'Finest ingredients only used, pure butter and highest quality flour and sugar; contains no drugs (except for diabetics).'

Applications for Driving Licences

One way out of the anomaly would be to add a provision to the section making it an offence to drive while knowingly suffering from a disease which is liable to cause unconsciousness—a provision which would throw a net much wider than necessary to catch a diabetic driver, as it would also cover anyone suffering from severe heart disease, high blood pressure or kidney disease. Perhaps a better way would be to prosecute such drivers under s. 112 (2) of the Road Traffic Act, 1930, for making a false statement in reply to question 12 or question 15 in the application for a driving licence; however, there is the difficulty of showing that such a statement was made 'knowingly' and also that the disability is 'likely' to be a source of danger to the public, which is difficult to prove especially in the light of the medical evidence which is likely to be adduced by the defence.

And in any case this does not really solve the problem of the diabetic driver, since justice does not, presumably, require that he should be prevented from driving altogether, but rather that the public should be protected from the dangerous manifestations of his disease and of its treatment. Justice does, however, demand that the diabetic driver should know precisely what his position is in law and that has been made rather more obscure by this last decision of the Divisional Court. This case has thrown into a dramatic highlight what has up to now been regarded as a mere matter of form—the filling in of licence applications—and solicitors will probably be asked to advise their 'ailing' clients on their position. In the present uncertain state of the law great caution should be exercised, and in doubtful

cases references should be made first to the client's doctor and then, if any doubt exists, by a discreet inquiry to the licensing authority.

[Margaret Puxon in *The Solicitors' Journal*, 16 February, 1957, p. 159.]

The Pre-Trial Conference

The University of California released a new film entitled *A Pre-Trial Conference* on 1 May 1957.

This new 25-minute 16 mm., black and white sound film will acquaint the legal profession with the pre-trial rules as adopted in California this year.

The pre-trial procedure was instituted in California after 7 years of intensive study and research.

It dramatizes a pre-trial conference based upon an exploding bottle case. The judge, the attorney for the plaintiff, and three defense attorneys perform roles appropriate to pre-trial situations.

The film opens with a brief explanation by the presiding judge of the purposes and aims of pre-trial conferences. In the discussion which follows, each attorney presents the theory of his client's case.

Some of the points covered are:

1. Medical examination of plaintiff.
2. Deposition and discovery.
3. Identification and admission of exhibits.
4. Limitation of number of experts.
5. Elimination of unnecessary parties.
6. Exploration of possible settlement.

The script was prepared by Clarence L. Kincaid, Judge of the Los Angeles County Superior Court and Chairman of the Committee on Pre-Trial of the Judicial Council of California. It was Judge Kincaid's Committee which prepared the pre-trial rules now in effect in California.

The action in the film clearly shows the sort of questions which can be handled in pre-trials and demonstrates the time-saving advantage of such conferences.

Prints of this new film may be rented for $7.50 (for a one-day showing with a graduated scale for more than one day). Purchase price: $100.00.

Further information can be obtained from:

Educational Film Sales Department,
University Extension,
University of California,
Los Angeles 24, California, U.S.A.